Field Evaluation of a Phototoxic Dye, Phloxine B, Against Three Species of Fruit Flies (Diptera: Tephritidae)

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ABSTRACT The xanthene dye phloxine B (D&C Red #28) bait was sprayed against fruit flies in mango orchards in 1996 and 1997. The flies used for testing were Mexican fruit fly, *Anastrepha ludens* (Loew), West Indian fruit fly, *Anastrepha obliqua* (Macquart), and Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Results of the experiments indicate that the toxic efficacy of phloxine B against these fruit flies is as good as that of malathion-bait sprays. Results also indicate that type of protein used with phloxine B can dramatically influence its efficacy. Hydrolyzed proteins of corn origin, Mazoferm 802 and Nutriplus, and one from microbial origin, Coltec yeast broth, were best. Phloxine B-bait applications as complete coverage or alternate swaths reduced fly populations as well as 19.5 or 9.8% (AI) malathion-Captor 300. Applications of phloxine B bait at concentrations of 0.12% phloxine B reduced populations as well as those applied at 0.48% (AI). The fruit fly parasitoid *Diachasmimorpha longicaudata* was adversely affected when exposed to phloxine B-Nutriplus bait but not when exposed to the other proteins.

KEY WORDS Mexican fruit fly, Mediterranean fruit fly, West Indian fruit fly, *Diachasmimorpha longicaudata*, phloxine B, phototoxic dye

PROBLEMS ASSOCIATED WITH the use of insecticides in bait-spray programs for fruit fly control such as public acceptance, ecological impact, and integration with pest management have been addressed in our laboratory by developing more precisely targeted bait systems that use insecticides which are less toxic to nontarget organisms. Bait and inorganic insecticide sprays such as lead arsenate and sodium fluosilicate to control fruit flies have been used since the beginning of the 20th century (Back and Pemberton 1918, Bodenheimer 1951). For example, one of Mally's mixtures was sugar, lead arsenate and water (Bodenheimer 1951). Post World War II hydrocarbon insecticides replaced inorganic compounds. The chlorinated hydrocarbon insecticides were replaced by organophosphates, which are used at present. According to Back and Pemberton (1918) baits for fruit fly control were first recommended in 1904 by Mally in South Africa against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and in 1908–1909 by Berlese in Italy against the olive fruit fly, *Bactrocera oleae* (Gmelin). They also reported that in about 1912 Lounsboury in South Africa improved the methodology for controlling *C. capitata* and in 1913–1914 Newman, in Australia, did the same to control the Queensland fruit fly, *Bactrocera tryoni* (Froggatt). About the same time, Marsh (1910) used low-volume insecticide applications against the melon fly, *Bactrocera cucurbitae* Coquillett, in Hawaii. Thereafter, other investigators adopted the low-volume approach to kill fruit flies. If baits were used, they added carbohydrates and fermenting substances such as sugars, syrups, or fruit juices. In the 1930s McPhail (1937), while working with attractants, found that sugar-yeast solutions attracted flies, and, in 1939 found that protein lures were attractive to *Anastrepha* species, especially to the guava fruit fly, *A. striata* (Schiner) (Baker et al. 1944). Steiner (1952) showed that hydrolyzed proteins or partially hydrolyzed yeast combined with organophosphate insecticides could control fruit flies. Protein hydrolyzate baits were first used in Hawaii to control the oriental fruit fly, *B. dorsalis* Hendel. The spray mixture contained protein hydrolyzate, sugar, and parathion (Steiner 1952). Shaw (1955) showed that a mixture of malathion and partially hydrolyzed yeast effectively controlled Mexican fruit fly, *Anastrepha ludens* (Loew), populations and the approach was adopted by USDA and the California Department of Agriculture. In 1956, malathion (an inhalation, contact, stomach poison) was combined with protein bait to eradicate *C. capitata* from Florida (Steiner et al. 1961). A mixture of technical grade malathion (≈ 95%) and NuLure (=Staley's Protein Insecticide Bait No. 7 or PIB-7) at a ratio

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of 1:4 (malathion:NuLure) was used to eradicate *C. capitata* from Brownsville, TX (Stephenson and McClung 1966). Lopez et al. (1969) also used malathion-bait mixed at the same ratio and applied to discrete locations on tree foliage to control Mexican fruit fly populations; however, they did not elucidate the rationale for their 1:4 ratio. Later Harris et al. (1971) showed that efficacy of malathion was directly proportional to the ratio of malathion to NuLure; a ratio of 1:4 was more effective than other ratios. Thus, flies responding to malathion bait may only need inhalation, contact or ingestion of mixture to die. This may be the reason why the adequacy of bait used in malathion-bait sprays was not questioned. The apparent reason for the wide use of malathion is its low mammalian toxicity, relative to other organophosphates and low price. However, ultra low-volume (1 liter/ha) sprays contain concentrations of technical malathion near 20% (AI).

A more aware public demands that insecticides such as organophosphates and carbamates be replaced with compounds less toxic to mammals and nontarget organisms and less injurious to the environment. Insecticides with novel modes of action would be desirable. Some photoactive dyes have been shown to have toxic properties to numerous insects (Heitz and Downum 1987, 1995). Dyes per se are not toxic but light energy can initiate a chain of toxic reactions in insect tissues. Among these dyes are arctic white TX, eosin Y, indigocarmine, erythrosine B, sunset yellow, ethylene blue, and sodium fluorescein (Lemke et al. 1987). Some of these dyes such as erythrosine B and phloxine B are food and/or drug and cosmetic additives (Green 1991). However, Krasnoff et al. (1994) were the first to show in laboratory tests that erythrosin B, was toxic to the tephritid apple maggot, *Rhagoletis pomonella* Walsh. Moreno and Mangan (1995) in laboratory tests showed that phloxine B was toxic to *A. ludens* and that the toxicity of phloxine B could be synergized with the use of the adjuvant SM-9. Mangan and Moreno (1995) obtained very similar results in field-cage studies.

To control fruit flies in the field with phototoxic dyes, a more accepting bait to fruit flies than NuLure was needed. The solution was a multicomponent bait that attracted flies, had acceptable texture, was phagostimulatory (attested by consumption), and potentially physiologically acceptable. Moreno and Mangan (1995) proposed a Mazoferm 802 bait formulation. The proposed bait consisted of 70% Mazoferm 802 for attraction and feeding, 20% invert sugar as a phagostimulant, 1% SM-9 as a penetrating adjuvant, 1% soybean oil as a stabilizer, 0.6% acetic acid for added attraction and preservation of bait, and optionally 0.4–1% xanthan gum as a thickening agent. Phloxine B was added to the Mazoferm bait and found toxic against *A. ludens.* Liquido et al. (1995a) studied the effect of phloxine B with uranine (1:1) or phloxine B alone with various proteins or with sugars against *C. capitata*, in the laboratory. They concluded that 100% female kill was achieved with ≈1% dye mixed with 20% autolyzed ICN yeast or 20% Mazoferm, with or without 20% fructose. Phloxine B did not perform as well in 10% molasses or 1% NuLure. Also, they found that by mixing 3.6% phloxine B and 7.1% methyl eugenol (1,2-dimethoxy-4-[2-propenyl]benzene), a potent attractant for males of *B. dorsalis*, they could kill flies in less than 2 h.

Table 1. Phloxine B-Mazoferm 802 formulation developed to kill fruit flies in the field

Ingredients	% Conc. (W, V/V)
Water, deionized	5.9
Phloxine B (92% [AI])	0.5
Polyethylene glycol 200	1.0
Invert sugar	20.0
Polysorbate 60	1.0
Soybean oil	1.0
Mazoferm 802	70.0
Acetic acid	0.6

The above formulation had an approximate pH of 3.5.

The objective of our study was to show that aerial applications of phloxine B properly formulated as a dye bait, could be used to suppress fly populations of *A. ludens, A. obliqua, and C. capitata* in Ataulfo mango orchards in the county of Tapachula, Chiapas, Mexico.

Materials and Methods

The formulation (Table 1) used in the field is very similar to that proposed by Moreno and Mangan (1995) and was tested in field-cage studies. The active ingredient is phloxine B (D&C Red #28; Warner Jenkinson, St. Louis, MO), a photoactive dye used in pharmaceuticals and cosmetics. The inert ingredients are already used in the formulation of foods or pharmaceuticals and cosmetics for human or animal consumption (see Lewis 1989, Smith 1991, and Budavari et al. 1996). Our rationale for using the ingredients was to provide an attractive, phagostimulatory, and stable formulation with acceptable texture, and nutritional qualities for a more rapid physiological assimilation by flies. Inert ingredients included polyethylene glycol 200 (Sigma, St. Louis, MO) used as a binder, dispersing agent, and texturizer for formulating foods and cosmetics. Invert sugar (mixture of fructose and glucose, Corn Products, Summit-Argo, IL) was used as a sweetener (phagostimulant), texturizer, humectant, and nutrient. Polysorbate 60 (polyoxyethene sorbitan monostearate, Sigma) was used as a penetrant adjuvant, an emulsifier, and stabilizer. Soybean oil was used as an emulsifier and texturizer. Mazoferm 802 (Corn Products) is a corn condensate hydrolyzed by a *Lactobacillus* sp. and consists of amino acids, vitamins, and minerals and is used as animal food. Acetic acid (Sigma) is concentrated vinegar and was used as preservative, acidifier, and flavoring. Acetic acid is used as an attractant (Moreno and Mangan 1995) and preservative in the Mazoferm bait. The other options for attraction were ammonium acetate or ammonium citrate but these compounds have no preservative properties and the attraction of flies to formulations at less than optimal pH is decreased considerably. The

LC_{50} of 240 mg/liter of phloxine B was determined by feeding *A. ludens* a mixture of 70% Mazoferm 802, 20% invert sugar, and 1% SM-9 (a nonionic adjuvant, SMI, Valdosta, GA). The only real modification to the proposed formulation was the change of adjuvant to the nonionic polysorbate 60 because it appeared to be a better penetrant in laboratory studies and is commonly used by food and cosmetics industries. In contrast, SM-9 was formulated as an agricultural adjuvant. In addition, the formulated dye bait does not alter any of the conventional methods of application.

1996 Experiments. Part of the objective of the 1996 experiments was to evaluate other available commercial proteins. These included Captor 300 (Pausa, Promotora Agropecuaria Universal, Mexico, D.F.) a hydrolyzed corn condensate very similar to NuLure (Miller Chemical & Fertilizer, Hanover, PA) and a Coltec yeast product (Grupo Coltec, Guatemala, Guatemala). We opted to use Captor 300 with malathion as our standard because it was the standard being used by the Moscamed Program in Mexico. Therefore, the treatments were: a Mazoferm 802 bait control, 0.5% (AI) phloxine B-Mazoferm bait, 0.5% phloxine B-Captor 300 bait, 0.5% phloxine B-Coltec yeast bait, and malathion (95% [AI]-Captor 300, 1:4 ratio, respectively). All formulations had the same ingredients, except malathion bait, differing only in protein used. Mazoferm and Captor 300 were used at a concentration of 70% by volume. The volume of Coltec yeast was adjusted to actual protein, ≈14%, as in the other two products. The concentration of phloxine B used in the field tests was 20.8 times the LC_{50} as determined against *A. ludens* in field cages. All the formulations were prepared 1 d before application. The applications were made with a Bell 206 helicopter equipped to spray ultra-low volumes. The helicopter was equipped with a boom and six evenly spaced #6 nozzles with orifice disk in place, three on each side of the helicopter's body. Line pressure was ≈2.1 kg/cm^2 During calibration spray droplets were measured in the range of 300–1,000-μm. Spray equipment was calibrated to deliver 4 liters/ha of dye-bait and malathion-bait was delivered at 1 liter/ha. The helicopter flew at speeds of 100 km/h and 30 m above ground laying a uniform spray swath ≈30 m wide. The spray application was total coverage. The sprays were conducted 25 May, 15 June, and 21 June 1996. About 12 h after the spray of 21 June it started raining and did not stop for 3 d. However, data obtained from this application were not excluded from analysis, nor materials reapplied.

Mango test plots were located in the coastal flatlands of the county of Tapachula, Chiapas, Mexico. The cultivar selected for the study was Ataulfo. All the plots were selected within a 200-ha mango orchard. Each plot comprised 10 ha and was isolated by a 300-m buffer area. A different colored flag (≈1/2 square meter) was used for each treatment and a flag was placed at each corner of a test plot on top of a bamboo mast 2–3 m above tree canopies. Each mast was tied to vertical branches with twine. Mango trees were between 5 and 7 yr old and experiments were conducted at the end of the harvest season. We used a randomized complete block design with three replications in space and three in time.

The phototoxic efficacy of phloxine B was evaluated against released, sterile *A. ludens* and *C. capitata.* Groups of flies emerged and fed on Wrappable Drier-70 (a dry sugary paste, Brokay Products, Philadelphia, PA) in paper bags in the laboratory for 3 to 4 d before release. Flies were hand-released from a helicopter from ≈500 m above ground. A technician ripped open a bag containing flies and threw it out the window and by the time the bag reached ground, almost all flies had flown away. About 3,000 flies of each species were released per ha. All fly releases were made between 0600 and 0900 hours CST, 24 h before applying treatments. The treatments were made between 0700 and 1100 hours. Traps to recapture surviving flies were put in place 48 h after spray applications. Two McPhail and two Jackson traps were used per hectare in each plot. McPhail traps were baited with one torula yeast tablet in 100 ml water, and Jackson traps were baited with 2 ml trimedlure on a cotton wick. Traps were evenly distributed in the core 6-ha sampling area, equidistant from each other, and placed at two-thirds height of the tree on the NE quadrant. Traps were left in place for 4 d then picked up, and recaptured flies were recorded.

1997 Experiments. Based on the results obtained in 1996, phloxine B was tested again at concentrations of 5, 10, and 20 times the laboratory LC_{50} against *A. ludens* and *A. obliqua.* The objectives were to determine the most effective formulation (based on protein) and concentration based on the efficacy of phloxine B sprayed against fruit flies in Ataulfo mango orchards. Also, an alternate swath application and the proteins Coltec yeast broth and Nutriplus (a hydrolyzed corn steepwater, Arancia Ingredientes Especiales, Celaya, Guanajuato, Mexico) were evaluated as baits. Thus, the treatments were control (Mazoferm 802 bait, complete coverage), 0.12% phloxine B-Mazoferm bait (complete coverage), 0.24% phloxine B-Mazoferm bait (complete coverage), 0.48% phloxine B-Mazoferm bait (complete coverage), 0.48% phloxine B-Mazoferm bait (alternate swath), 0.48% phloxine B-Nutriplus (complete coverage), 0.48% phloxine B-Coltec yeast broth (complete coverage), and malathion 95% (AI)-Captor 300 (1:9 respectively, complete coverage). The proteins Mazoferm 802, Nutriplus, and Coltec yeast broth were used at 70% by volume. All treatments were applied at 3.8 liters/ha except the alternate swath treatment which was applied at 1.9 liters/ha and malathion-bait at 1 liter/ha on 28 March, and 4, 13, and 21 April 1997. The same procedures used in the 1996 experiments were employed in this test. The differences were the number of repetitions (four in space and four in time), only McPhail traps were used for fly recapture, and fly releases were made via ground (ripping paper bags from a moving vehicle). In addition, an attempt was made to obtain a larger spray droplet size (≈4,000 μm) than the previous year (average 400 μm).

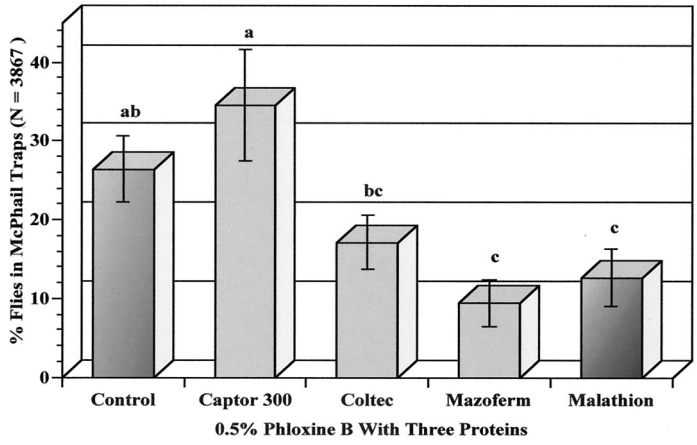

Fig. 1. Effect of phloxine B with the proteins Captor 300, Coltec yeast, and Mazoferm 802 as compared with a malathion-Captor 300 and a Mazoferm bait control against released, sterile Mexican fruit fly, *A. ludens*, Tapachula, Chiapas, Mexico, 1996

All applications were made via helicopter (Bell Jet 206). The boom in this case had four #6 nozzles, two on each side of the helicopter; each nozzle was wide open and had an 8-cm stainless steel tube extension welded onto it. The tube's i.d. was 4.8 mm. Line pressure ≈2.1 kg/cm^2. These changes were made in an attempt to deliver larger droplets than those delivered in 1996. The nozzle modification allowed formulation discharge in a uniform tubular stream. The stream was uniform for ≈2.5 cm then wind speed rotated the stream creating a horizontal vortex for ≈8 cm bending down gradually and split by wind shear ≈15 cm from the discharge orifice. Most large droplets landing on foliage ranged from 4,000 to 6,000 μm in diameter.

In addition to the above tests, we assayed the susceptibility of the braconid fruit fly parasitoid *Diachasmimorpha longicaudata* to the dye treatments made above, excepting the malathion and alternate swath treatments. Thus, the treatments consisted of control, 0.12% phloxine B-Mazoferm, 0.24% phloxine B-Mazoferm, 0.48% phloxine B-Mazoferm, 0.48% phloxine B-Nutriplus, and 0.48% phloxine B-Coltec yeast broth. We took a dye-bait sample from the field to the laboratory to conduct this test. For this we used a plastic cage constructed of multiple units. The first was a polypropylene bottle (250-m-wide mouth) used to provide drinking or sugar-water. The second consisted of a supporting ring glued to the water bottle. The third was a butyrate ring covered with a white, polyester veil having hexagonal 1-mm openings. The fourth was a round Teflon rod trough in which food was placed. Parasitoids were obtained from the rearing facility located at San Miguel de Metapa, Chiapas. Twenty female parasitoids were transferred into each cage through one end of the holes made for the Teflon rod. Parasitoids in cages were prepared early in the afternoon and had a choice of feeding on sugar-water only or an experimental dye-bait for the rest of the day before lights were turned off. Early the next morning, all cages were taken outside and exposed to direct sunlight. An initial reading was made to determine overnight mortality and every hour thereafter until solar radiation started killing parasitoids in the control treatment.

Statistics. All fly recapture data were analyzed using a two-way analysis of variance (ANOVA) with a generalized linear model statistical (SuperAnova) package (Abacus Concepts, Berkeley, CA). All fly recapture data were logged as percentages for ease of comparison among repetitions in time. All data, except parasitoid data, were normalized with the square root of x plus 0.5 transformation before analysis, but data are presented in percentages as logged.

Results

1996 Experiments. The 1996 results indicated the efficacy of phloxine B-Mazoferm worked as well as malathion-Captor 300 in reducing fly populations (Figs. 1 and 2). Results of the ANOVA indicated that the spray treatments were having a significant negative impact on *A. ludens* ($F = 6.85$; df = 4, 37; $P < 0.001$). Results of the least significant difference (LSD) test ($P = 0.05$) indicated that phloxine B-Mazoferm and malathion-bait spray were significantly more effective at controlling *A. ludens* than Captor 300 treatments (Fig. 1). The efficacy of phloxine B with Coltec yeast broth appears to be acceptable to flies and could only be significantly separated from phloxine-Captor 300 spray. The efficacy of phloxine B with Captor 300 indicates that this protein has something in its composition that is not accepted by flies or that phloxine B reacts with some chemical in the product. Results of ANOVA also indicated a significant negative impact of sprays against *C. capitata* ($F = 4.36$; df = 4, 37; $P = 0.006$). The profile of results with *C. capitata* was similar to that of *A. ludens* (Fig. 2). Results of the Fisher Protected LSD mean separation at 0.05 level of probability indicated that the impact of phloxine B-Coltec sprays could not be separated from control or

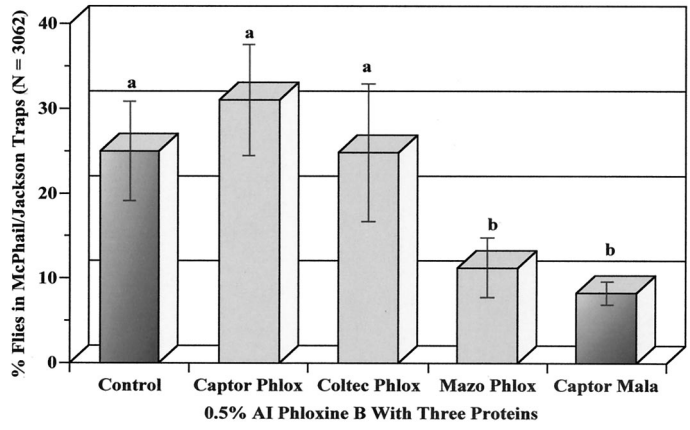

Fig. 2. Effect of phloxine B with the proteins Captor 300, Coltec yeast, and Mazoferm 802 as compared with a malathion-Captor 300 and a Mazoferm bait control against released, sterile Mediterranean fruit fly *C. capitata*, Tapachula, Chiapas, Mexico, 1996.

phloxine-Captor 300 sprays or the other treatments. However, there was a significant difference between means for phloxine B-Mazoferm and malathion-Captor 300 and those from the control and phloxine B-Captor 300; there is no significant difference between means of phloxine B-Mazoferm and malathion-Captor 300. There was no significant interaction between spray replicates and dates in the applications for both species.

1997 Experiments. The 1997 results indicated that phloxine B was as efficacious in reducing populations of flies as a malathion-bait spray. Results of the ANOVA indicated a significant impact of sprays on *A. ludens* ($F = 12.52$; df = 7, 105; $P < 0.001$). However, there was also a significant interaction between spray replicates and dates ($F = 3.92$; df = 15, 105; $P = < 001$). According to the Fisher Protected LSD mean separation at 0.05 level of probability, all treatment means were significantly different from the control and all phloxine B applications were as efficacious in reducing *A. ludens* populations as malathion-Captor 300 (Fig. 3). The results for the 0.48% phloxine B-Mazoferm alternate swath spray were as good as a complete coverage application with the same ingredients and that of malathion-Captor 300 sprays. Also, there was no significant difference in reducing fly populations among the proteins Coltec yeast broth, Nutriplus, or Mazoferm with 0.48% phloxine B. Results of the ANOVA also indicated a significant negative impact of sprays on *A. obliqua* ($F = 7.14$; df = 7, 105; $P < 0.001$). Again, there was a significant interaction between replicates and spray dates ($F = 7.51$; df = 15, 105; $P = < 001$). Nonetheless, all spray applications reduced *A. obliqua* populations compared with the control, according to the Fisher protected LSD mean separation at 0.05 level of probability (Fig. 4). Also, there were no significant differences between treatments. The response of *A. obliqua* to spray applications was very

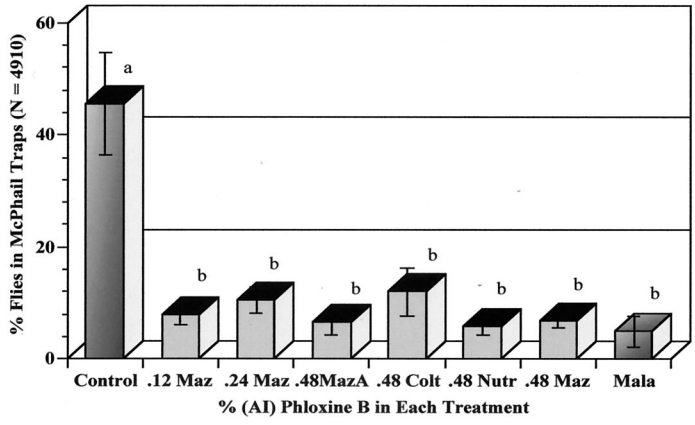

Fig. 3. Effect of the phototoxic dye phloxine B sprayed at various concentrations against Mexican fruit fly populations, as compared with a Mazoferm bait control and a malathion-Captor 300 standard. The sprays were applied as uniform coverage in Mazoferm (Maz), Coltec yeast (Colt), and Nutriferm (Nutr) and alternate swaths (MazA) via helicopter, in Ataulfo mango orchards. Tapachula, Chiapas, Mexico, 1997.

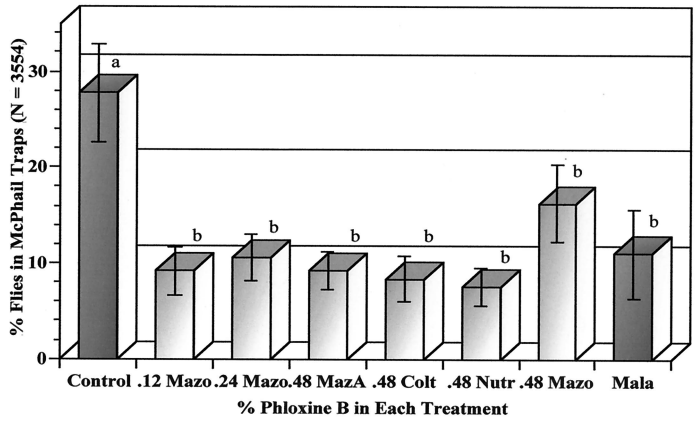

Fig. 4. Effect of the phototoxic dye phloxine B against West Indian fruit fly, *A. obliqua*, populations, as compared with a Mazoferm bait control and a malathion-Captor 300 standard. The sprays were applied as uniform coverage in Mazoferm (Maz), Coltec yeast (Colt), and Nutriferm (Nutr) and alternate swath in Mazoferm (MazA) via helicopter, in Ataulfo mango orchards. Tapachula, Chiapas, Mexico, 1997.

similar to *A. ludens.* The 0.48% phloxine B-Mazoferm alternate swath spray application reduced fly populations as well as a complete coverage spray using the same ingredients. The efficacy of proteins in Coltec yeast broth, Nutriplus, and Mazoferm was similar. The efficacy profile for phloxine B was very similar for both species of fruit flies, indicating similar acceptability of the Mazoferm bait formulation.

Results with the parasitoid *D. longicaudata* indicated that this parasitoid fed on the formulation of 0.48% phloxine B-Nutriplus and was adversely affected (Fig 5). ANOVA shows that significant mortality of the parasitoid occurred with the treatments ($F = 21.72$; df = 5, 20; $P < 0.001$). According to the Fisher Protected LSD mean separation at 0.05 level of probability, there were no significant differences between treatment means except for 0.48% phloxine B-Nutriplus. Mortality of the parasitoid occurred in a period of 3 h under direct sunlight exposure.

Discussion

The profile of information obtained in the 1996 experiments with *A. ludens* and *A. obliqua* indicates that the two species are responding similarly to phloxine B and malathion-Captor 300 bait sprays. The phototoxic dye phloxine B is equally toxic to malathion; however, there is a great difference in safety margin. The oral LD_{50} in rats for malathion is 1,375 mg/kg (Budavari et al. 1996) whereas phloxine B is calculated to be over 100,000 times safer than malathion (Heitz and Downum 1995). According to Bergsten (1995), phloxine B in practice is not anticipated to persist or remain toxic for very long in the environment; phloxine B is photodegradable with a half-life of ≈1 h and the compound has a very low affinity for lipids. Thus, bioaccumulation should be minimal. Captor 300 used at 80 and 90% with 19.5 and 9.8% (AI) malathion, respectively, and at 70% with phloxine B, appears to

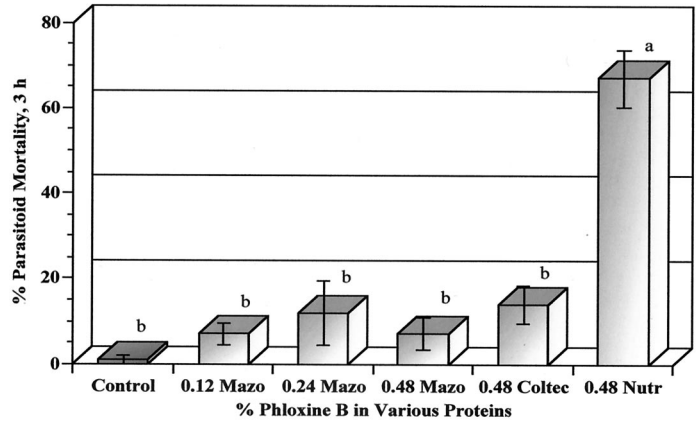

Fig. 5. Mortality of the braconid fruit fly parasitoid *D. longicaudata* exposed to bait containing varying concentrations of the phototoxin phloxine B in various protein-baits. Tapachula, Chiapas, Mexico, 1997.

work well with malathion but not with phloxine B at 0.5% (AI). Malathion is an inhalation, contact, and stomach poison but phloxine B is classified only a stomach poison. Therefore, many of the flies could be killed by inhalation or by contacting malathion-bait. This type of poisoning could not occur with phloxine B because its effect is only through ingestion. Phloxine B is not a poison per se but its presence in living tissues (in conjunction with the sun's energy) releases free oxygen which reacts with phospholipids, enzymes, and cellular organelles that interfere with intra- and intercellular communication. These effects render the organism incapable of performing normal physiological functions (Heitz 1995). When this happens, death is imminent. However, there was a much greater survival of flies in plots treated with phloxine B-Captor 300 than with any of the other treatments. Thus, indicating that flies were eating some of the phloxine B-Captor 300 mixture but not enough to kill them or the efficacy of phloxine B was severely reduced by a compound(s) in the mixture. Our understanding is that Captor 300 is produced through the process of concentrated acid hydrolysis of corn steepwater similar to NuLure; in which case, up to ≈16% salt is produced and the product has a pH of ≈3.9–4.0. In addition, Captor 300 is neutralized and its pH raised to ≈7.7 to make the protein more attractive to flies (Anonymous, undated pamphlet); this process produces more salt. High concentrations of salt are not natural to phytophagous insects and the reaction could be to avoid salty products but to eat just enough to survive. Ostensibly, the inclusion of 20% invert sugar was not sufficient to completely attenuate the saltiness of the formulation. This could be the case with phloxine B-Captor 300 mixture. In addition, raising the pH to 7.7 creates an inimical environment for malathion which is easily hydrolyzed above pH 7 and below pH 5 and it is most stable at pH 5.26 (Budavari et al. 1996).

In the second set of experiments in 1997 with *A. ludens and A. obliqua*, results were much more definitive. The data suggest that the concentration of phloxine B can be lowered to five times the accepted LC_{50} and still control fly populations as well as malathion-Captor 300. Also, the proteins Coltec yeast broth, Nutriplus, and Mazoferm performed well with phloxine B, and at least in the case of Coltec yeast broth, much better than in the 1996 experiments. Not too surprising, the alternate swath spray with 0.48% phloxine B-Mazoferm performed as well as a complete coverage spray with same ingredients and as well as a malathion-Captor 300 spray. The explanation may be in the significantly larger droplet size (≈4–6 mm diameter) obtained in 1997 than those produced (≈0.4 mm diameter) in 1996. The larger droplet would tend to stay as liquid or soft syrup much longer than smaller droplets in the dry flatlands of coastal Chiapas, Mexico, where the experiments were conducted. Thus, larger droplets may retain attraction and availability to fruit flies through more persistent syrupy texture, provided by the hygroscopic properties of invert sugar and polyethylene glycol 200. Whether the efficacy of the alternate swath treatment is due to the larger droplet or the attraction of the bait, does not matter much in practice if fly control is the same as with complete coverage applications. From an economic point of view, it would be cheaper to spray only half of the area; and from an ecological perspective, it would give nontarget organisms an opportunity to escape to untreated refugia or allow them time to recover from the adversity of a spray application. We have no explanation for the decreased mortality observed with 0.48% phloxine B-Mazoferm and *A. obliqua* in 1997. We also did not notice decreased fly kill in 1997 despite using 1/2 the malathion used in 1996. This may be because malathion is already used in excessive concentrations to kill flies. Thomson (1998) lists the high-end recommendation to control insects as 0.24% or 2,400 mg/liter (AI), which is 81-fold less than the one recommended to eradicate flies. As a general rule, once an LC_{50} is found on a given insect in the laboratory, the figure is multiplied by a factor of 100 for field use; this multiple should temporarily overcome adversities such as UV degradation and high pH (≤8) water found in practice. In our case, we used a field concentration of 20 times the LC_{50} and were able to show significant fly reduction compared with the control. This implies that the dye-bait persists long enough for most flies to feed and be affected by the photo-oxidative properties of phloxine B. Before these results, no one had shown any control of fruit flies in the field, except in field-cage studies (Mangan and Moreno 1995). However, Pimprikar et al. (1980) were able to reduce house fly populations in small- and large-scale field tests with the dye erythrosin B but Burg et al. (1989) did not have similar success. Other attempts to control fruit flies in the field with less toxic insecticides such as cyromazine (Moreno et al. 1994, Diaz et al. 1996) and boric acid-borax mixtures (Enkerlin et al. 1993) showed some success. Perhaps results would have been better if they had used more palatable bait than NuLure, such as the Mazoferm we used. Moreno et al. (1994) noted that phagostimulation of *A. ludens* increased with the addition of 20% fructose in NuLure but the cyromazine-NuLure applications in the field were made without any sugar.

The response of *D. longicaudata* to phloxine B baits was predictable for the phloxine B-Mazoferm formulation, based on previous laboratory observations, but surprisingly the response to phloxine B-Nutriplus was not expected because it is a very similar protein to Mazoferm 802. Both proteins are derived from corn steep condensate but the production process may be different. However, even though these products are similar this does not mean they are homologous and consequently parasitoids found something attractive and acceptable in the Nutriplus bait that was not found in either Mazoferm or Coltec yeast broth bait. Outside of proteins, the formulations were the same. This information indicates that care should be taken in the selection of protein baits because they may be attractive and acceptable to nontarget or beneficial species. One should be watchful even with the use of proteins; preferably, one should choose a protein that accom-

plishes the objective of attracting and feeding flies but should not do the same to beneficial organisms. It would be preferable to exclude beneficial organisms through their gustatory behavior. There is room for bait improvement and better attractants other than acetic acid that should be evaluated for their attractiveness to beneficial organisms such as fly parasitoids and bees. Ammonium acetate and ammonium citrate, in higher pH baits, among others should be evaluated for this purpose.

Phloxine B kills only through ingestion of the active ingredient. According to these results, the phloxine B concentration can be reduced down to 0.12% and would reduce costs. If the costs of various ingredients in the formulations are compared and only the cost of proteins is changed, the least expensive formulation is with Mazoferm. The cost of this formulation is very favorable compared with the current cost (US$) of spraying malathion-bait by air. Furthermore, phloxine B has a social benefit that is difficult to measure in monetary terms but meets the objective of social consciousness, to maintain natural ecosystems, and safeguard the environment. Overall the results of our field experiments with the phototoxic dye phloxine B (D&C Red #28) indicate that the toxic efficacy of phloxine B is as good as that of a malathion-bait spray against *A. ludens, A. obliqua,* and *C. capitata.* Results also indicate that type of protein used with phloxine B can dramatically influence its efficacy.

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